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(54) Title: ISOFLAVONES FOR TREATING GIARDIASIS AND MALARIA

(57) Abstract

The present invention relates to a pharmaceutical composition and method of treating giardiasis and/or malaria by administering to a subject in need of such treatment an effective amount of at least one compound such as formononetin, pseudobaptigenin and other isoflavones.

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ISOFLAVONES FOR TREATING GIARDIASIS AND MALARIA

FIELD OF INVENTION

The present invention relates to antigiardial activity of formononetin, pseudobaptigenin, and other isoflavones and their formulations.

BACKGROUND OF INVENTION

Giardia lamblia (or G. intestinalis) is a flagellated protozoan parasite which is the most frequent cause of intestinal protozoal infections in the world (Hill, D.R. Giardiasis: issues in diagnosis and management. Infect. Dis. Clin. North Am. 7:503-525, 1993 and Farthing, M.J.G. "Giardiasis as a Disease" in Giardia: From Molecules to Disease, eds. R.C.A. Thompson, J.A. Reynoldson, and A.J. Lymbery, CAB International, Wallingford, UK, 1994, pp. 15-37), and the most frequent cause of non-bacterial diarrhea in North America (U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition. Foodborne Pathogenic Microorganisms and Natural Toxins 1992 (Bad Bug Book). In some parts of the world, 20-30% of the population is affected (Farthing, J.J.G. "Giardiasis as a Disease" in Giardia: From Molecules to Disease, eds. R.C.A. Thompson, J.A. Reynoldson, and A.J. Lymbery, CAB International, Wallingford, UK, 1994, pp. 15-37). The disease is especially prevalent in infants and children in the developing world, and it can have devastating effects due to malabsorption and malnutrition. Most transmission is via contaminated water, but some outbreaks have been traced to improperly prepared foods.

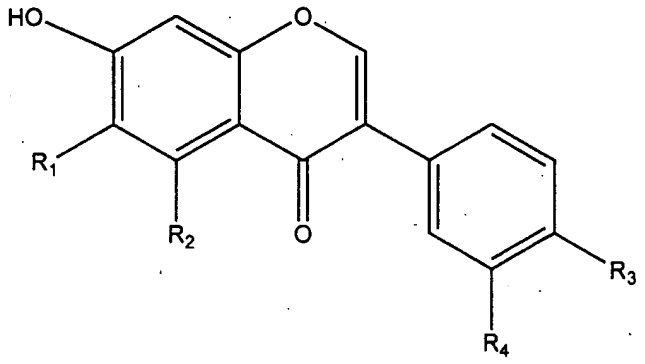
Three classes of drugs are currently utilized for treatment of giardiasis: metronidazole and derivatives; mepacrine and analogs; and nitrofurans such as furazolidone. Metronidazole is most widely used, and is generally effective and well-tolerated. However, treatment failures have occurred in up to 20% of patients, and reports of resistance have appeared (Johnson, P.J. Metronidazole and drug resistance. Parasitol. Today, 9:183-186, 1993). In addition, the toxicity of metronidazole is notable (Roe, F.J.C. Metronidazole: review of uses and toxicity. J. Antimicrob. Chemother. 3:205-212, 1977), with gastrointestinal upset, headache, nausea, and

unpleasant taste commonly reported. Metronidazole has also been associated with more serious neurologic side effects - convulsions, paresthesias, ataxia, encephalopathy. The reported carcinogenic and mutagenic effects of the drug, while not established in the clinic, have raised further concerns about safety. Mepacrine and furazolidone both have serious toxicities as well, so that there is a need for effective and safer agents for the chemotherapy of giardiasis.

SUMMARY OF THE INVENTION

The present invention of antigiardial activity of formononetin, pseudobaptigenin, and related compounds began with the initial screening of several extracts of the bark of Dalbergia frutescens, obtained from the plant collection of the National Center for the Development of Natural Products.

The invention claimed is for the use of formononetin, pseudobaptigenin, and synthetic or natural derivatives of this structural class of compounds, with substitutents at (R1-R4 or combinations thereof) in the therapy of giardiasis. The compounds have the following formula I:



in which R1, R2, R3 and R4 are each independently H, OH or lower alkoxy or R3 and R4 are taken together to form a O-CH2-O bridge. Lower alkoxy includes C1-C3 alkoxy and OCH3 is preferred.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of the compounds of formula I, above as antigiardial compositions. In a preferred embodiment of the present invention, R_1 is H, OH or lower alkoxy, R_2 is H or OH, R_3 is H, R_4 is H or lower alkoxy or R_3 and R_4 are taken together to form a O-CH_2-O bridge.

Furthermore, the invention includes various formulations of said compounds of formula I suitable for oral administration; various salt forms and/or prodrugs of the said compounds suitable for oral administration; the compounds, their salts or prodrugs can be prepared as elixirs and suspensions in sterile aqueous vehicles and also can be presented admixed with binder, carriers, diluents, disintegrants and the like as powders, as pills, or as capsules. Typical salt forming ions include, for example, alkali metal, ammonium or tetrakeylammonium. Suitable alkali metals include sodium or potassium. The compounds of formula I can be targeted for delivery to the intestine by prodrug formation such as to a polymeric material, or by incorporation into a hydrogel. Typical liquid vehicles include polyvinylpyrrolidone, N-methylpyrrolidone, sterile water and sterile sugar syrup. Typical solid materials include starch, destrose, mannitol microcrystalline cellulose and the like. typical prodrugs include glucopyranosides of a functional group such as a phenolic group, esters, carbonates, and urethanes. Typical polymeric materials include polyethylene glycol or N-(2-hydroxypropyl)methacrylamide copolymer and the like, and may be either entrained into the polymer, or may be covalently attached by the functional groups mentioned above.

Pharmaceutical composition within the scope of the present invention comprise at least one compound of formula (I) or a pharmaceutically acceptable salt or prodrug form thereof and a pharmaceutically acceptable carrier. The pharmaceutical composition may be in the form of a botanical, phytomedicine, nutraceutical or dietary supplement.

The actual dosage amount administered can be determined by physical and physiological factors such as body weight, severity of condition, and idiopathy of the patient. With these considerations in mind, the dosage of compounds of formula I

and formulations containing those compounds for a particular subject and or course of treatment can readily be determined.

Example 1

The pulverized bark of Dalbergia frutescens was extracted successively with hexane, 50% hexane/ethyl acetate, ethyl acetate, and 95% ethanol at 40 deg C with overhead stirring. All 4 extracts of the original plant material showed activity in the original screening at <56 μg/ml of the crude extract. Confirmation and further evaluation showed the highest activity in the Hexane/EtOAc and EtOAc extracts (8 and 11μg/ml of crude extract, respectively). These two extracts were combined for chemical investigation.

The combined extract was chromatographed over silica gel (column chromatography), eluting with increasing polarity from 20% ethyl acetate-toluene to ethyl acetate and then washed with methanol. Fractions (640 total) were collected and pooled according to their thin layer chromatographic patterns; bioactivity in antigiardial screening showed nine active pooled fractions (30C- 30K), which were rechromatographed over silica gel using a step gradient of $CHCL_3$ in methanol. The active fractions were further resolved and finally purified by RP-HPLC (Ultracarb 5 ODS 30, 250X10 mm, Phenomenex), eluting with 50% MeOH/H20().8% TFA), to obtain compound **1** (IC_{50} <0.56 μg/ml); structures are depicted in **Table 1**. The structures of the active compounds were determined by mass spectrometry, ^1H and ^{13}C-nmr spectral data analysis and comparison with previously reported data (Murthy, MSR et al., Magn. Reson. Chem., 1986, 24, 255; Murthy, MSR et al., J. Nat. Prod. 1985, 48, 967; Wenkert et al., Phytochemistry, 16, 1977,1811; Markham et al., Tetrahedron, 32, 1976, 2607; Rao, EV et al., Phytochemistry, 1985, 24, 875).

ANTIGIARDIAL ASSAY

Giardia intestinalis (ATCC 30888) was grown in Keister's modified TYI-S-3 medium under nitrogen at 37 deg C. For assay, 100 ml of Keister's medium containing *Giardia* cells, at a concentration of 1,000,000 per ml, were added to each well of a Corning 96-well microtiter plate. The volume in each well was brought up

to a total volume of 250 ml by adding the appropriate amount of Keister's medium. The plates were incubated in a modular incubator under nitrogen for 24 hours at 37 degrees C.

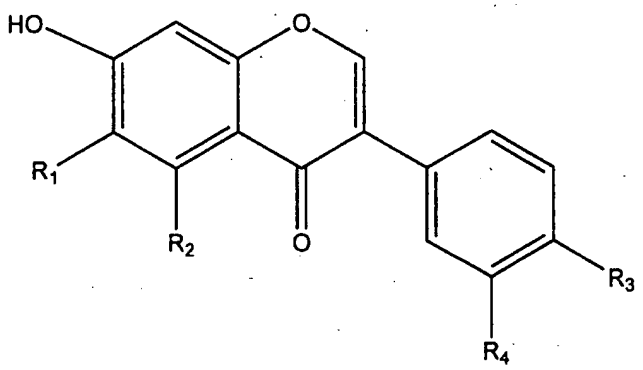
At 24 hours, 50 µl of the crude extract serial dilutions were added to each well in duplicate at final concentrations of 500, 166, and 56 µg/ml. Blanks and vehicle controls were also included in each assay. The total volume in each well was 300 µl. The plates were incubated for an additional 24 hours.

The viability of the *Giardia* was determined using a modified tetrazolium salt method with XTT (Wright et al, 1992). The Keister's medium was discarded from the plates and each plate was rinsed with warm saline. 100 µl of PBS containing 1% dextrose was added to each well. Then to each well 25 µl of the XTT solution was added. The plates were incubated for 4 hours at 37 degrees C, and read on a Bio-tek EL312 plate reader at 450mm, with background at 630 nm subtracted from the readings.

Mammalian cell cytotoxicity was simultaneously estimated with a cell growth assay using Vero cells (ATCC CCL81). Viability of the cells was determined with a neutral red satin, with a modification of the method of Borenfreund et al (1986).

The results of this assay for the instant compounds are shown in Table 1.

Table 1. *In Vitro* IC_{50} Values (μg/mL) of Isoflavone Antigiardials).



Structure	R1	R2	R3	R4	IC_{50}
1	H	H	H	OCH_3	0.03
2	H	H	H	H	3.75
3	H	OH	H	OCH_3	3.50
4	OH	H	H	H	>5.0
5	OCH_3	H	H	OCH_3	>5.0
6	H	H	$O\text{-}CH_2\text{-}O$		<0.56
7	OCH_3	H	$O\text{-}CH_2\text{-}O$		1.5

DISCUSSION OF RESULTS

The two most active compounds were formononetin **1** and pseudobaptigenin **6**, with IC_{50} values of 0.03 μg/ml and, 0.56 μg/ml (the IC_{50} of pseudobaptigenin was not titrated down below 0.56 μg/ml). The only difference between **1** and **6** is that pseudobaptigenin contains a methylenedioxy ring attached to the C-ring where formononetin is mono-substituted in ring C with only a methyl group.

The antigiardial activity of formonetin is shown in **Figure 1**. The 48 hr IC_{50} of formononetin is approximately 0.03 μg/ml (0.1 μM), compared to metronidazole, which has an IC_{50} of approximately 0.1 μg/ml (0.6 μM) in this assay.

In time course studies, formononetin impaired motility and reduced viability of *Giardia* at 2 and 4 hours of exposure (IC_{50} < 1 μg/ml), as contrasted with metronidazole which has no effect on *Giardia* cultures at these time points. Thus, formononetin demonstrated antigiardial activity comparable to compounds used for treatment of giardiasis, such as metronidazole and derivatives, mepacrine and analogs, and nitrofurans such as furazolidone. Furthermore, formononetin is non-toxic to mammalian cells at concentrations up to 50 μg/ml.

Table 2 summarizes the results of an evaluation of the *in vivo* efficacy of formononetin in the treatment of giardiasis in a mouse model. Mice were treated with a single daily dose of 1 mg or 10 mg by oral gavage for 7 days, beginning on day 3 after inoculation. These studies were conducted at the National Institute of Allergy and Infectious Diseases, NIH, in the laboratory of Dr. Theodore Nash.

Treatment	**Infected/Treated**	**Organisms/HPF**
Control 1	10/10	8.9
Treated (1 mg/day)	10/10	8.4
Treated (10 mg/day)	2/10	0.625
Control 2	9/10	9.7
Vehicle (DMSO:surfactant: water, 1:1:8)	10/10	9.0

The above examples are presented as specific and preferred embodiments. Although the invention has been described in conjunction with specific embodiments, it is evident that many alternatives and variations will be apparent to those skilled in the art. Accordingly, the present invention is intended to embrace all alternatives and variations that fall within the spirit and scope of the appended claims. All references and citations mentioned in this disclosure are hereby incorporated by reference.

LITERATURE:

Hill, D.R. Giardiasis: issues in diagnosis and management. *Infect. Dis. Clin. North Am.* 7:503-525, 1993.

Johnson, P.J. Metronidazole and drug resistance. *Parasitol. Today*, 9:183-186, 1993.

Farthing, M.J.G. "Giardiasis as a Disease" in Giardia: From Molecules to Disease, eds. R.C.A. Thompson, J.A. Reynoldson, and A.J. Lymbery, CAB International, Wallingford, UK, 1994, pp. 15-37.

U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition. Foodborne Pathogenic Microorganisms and Natural Toxins 1992 (Bad Bug Book).

Roe, F.J.C. Metronidazole: review of uses and toxicity. *J. Antimicrob. Chemother.* 3:205-212, 1997.

Wright, C.W., S.I. McIwani, J.D. Phillipson, and D.C. Warhurst. Determination of anti-giardial activity in vitro by means of soluble formazan production. *Trans. Roy. Soc. Trop. Med.* 86:517-519, 1992.

Borenfreund, E., H. Babich, and N. Martin-Alguacil. *In vitro Dev. Cell. Biol.* 26:449, 1986.

Murthy, MSR et al., *Magn. Reson. Chem.*, 1986, 24, 255.

Murthy, MSR et al., *J. Nat. Prod.* 1985, 48, 967.

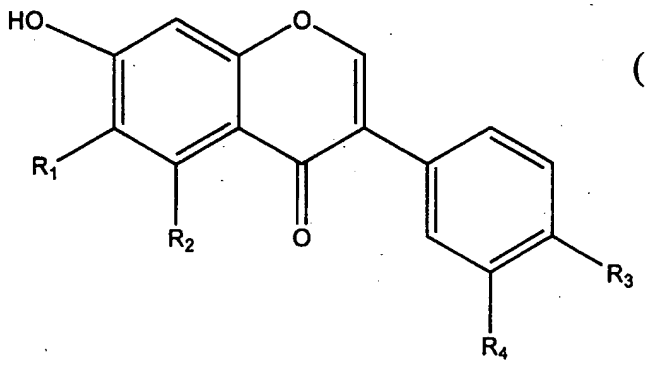
Rao, EV et al., *Phytochemistry*, 1985, 24, 875.

Wenkert et al., *Phytochemistry*, 16, 1977, 1811.

Markham et al., *Tetrahedron*, 32, 1976, 2607.

WE CLAIM:

1. A method of treating giardiasis and/or malaria comprising administering to a subject in need of such treatment an effective amount of at least one compound having the formula I:



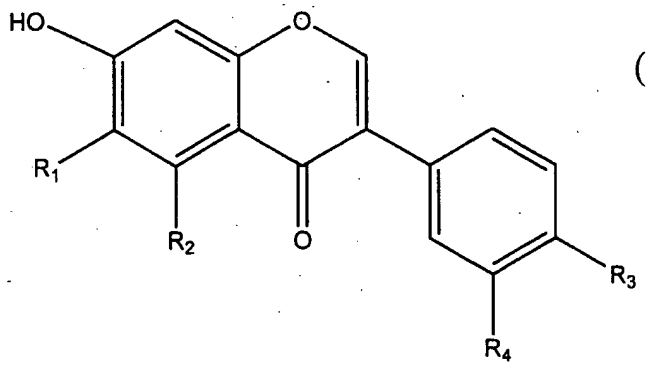
(I)

Wherein R1, R2, R3, and R4 are each independently hydrogen or alkoxy or R3 and R4 are taken together to form a dioxolane bridge, O-CH2-O.

2. The method according to claim 1, wherein R_1 is H, OH or lower alkoxy, R_2 is H or H, R_3 is H, R_4 is H or lower alkoxy or R_3 and R_4 are taken together to form a O-CH_2-O bridge.

3. The method according to claim 1, wherein the salt forming basic ion is sodium, potassium, or tetraalkylammonium.

4. A pharmaceutical composition for the treatment of giardiasis or malaria comprising at least one compound having the formula I:



(I)

wherein R_1, R_2, R_3, and R_4 are each independently hydrogen, hydroxy or alkoxy or R_3 and R_4 are taken together to form a dioxolane bridge, O-CH_2-O or pharmaceutically acceptable salt or prodrug form thereof and a pharmaceutically acceptable carrier.

5. The composition according to claim 4, wherein R_1 is H, OH or lower alkoxy, R_2 is H or H, R_3 is H, R_4 is H or lower alkoxy or R_3 and R_4 are taken together to form a O-CH_2-O bridge.

6. The composition according to claim 4 salt forming basic ion is sodium, potassium or tetraalkylammonium.

7. The composition according to claim 4, wherein the composition is suitable for oral administration.

8. The composition of claim 4 in the form of a botanical, phytomedicine, nutraceutical, or dietary supplement useful in the treatment of giardiasis.

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/US 99/06904

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/35 A61K31/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 507 256 A (SQUIBB BRISTOL MYERS CO ;SEATTLE BIOMEDICAL RESEARCH IN (US)) 7 October 1992 see claims 1,2,5,8,9,12 ---	1-8
X	A.R. DLUZEWSKI: "Inhibition of invasion and intraerythrocytic development of Plasmodium falciparum by kinase inhibitors" EXPERIENTIA, vol. 52, no. 6, 1996, pages 621-623, XP002106743 see the whole document ---	1,4,7,8
X	WO 93 23069 A (KELLY GRAHAM EDMUND) 25 November 1993 see claims ---	4-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 1999	06/07/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/US 99/06904

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 23716 A (UNIV TUFTS MED) 27 October 1994 see the whole document	4-8

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/06904

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-3
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-3 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 99/06904

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0507256 A	07-10-1992	CA 2064550 A	02-10-1992
		JP 5132429 A	28-05-1993
WO 9323069 A	25-11-1993	AU 683838 B	27-11-1997
		AU 4052593 A	13-12-1993
		BR 9306393 A	15-09-1998
		CA 2136233 A	25-11-1993
		EP 0656786 A	14-06-1995
		JP 7506822 T	27-07-1995
		NO 944435 A	18-11-1994
		NZ 252051 A	28-10-1996
		US 5830887 A	03-11-1998
WO 9423716 A	27-10-1994	AU 6635994 A	08-11-1994
		CA 2160371 A	27-10-1994
		EP 0693927 A	31-01-1996
		US 5498631 A	12-03-1996